

**What is Claimed is:**

1. A method of evaluating clotting activity comprising:
  - (a) combining a sample comprising blood or plasma with:
    - (i) a soluble phospholipid;
    - (ii) a contact activator; and
    - (iii) calcium;
  - (b) incubating the mixture of (a) above for a time and under conditions sufficient for thrombin activation; and
  - (c) detecting Factor X<sub>a</sub> or thrombin activity, wherein the activity of Factor X<sub>a</sub> or thrombin is indicative of clotting factor activity in the sample.
2. The method of Claim 1, wherein the sample is from a subject with lupus.
3. The method of Claim 1, wherein the sample is further combined with Activated Protein C or a Protein C activator, wherein the level of thrombin activity is indicative of Activated Protein C resistance in the sample.
4. The method of Claim 3, wherein the sample is further combined with Protein S depleted plasma, wherein the level of thrombin activity is indicative of Protein S levels in the sample.
5. The method of Claim 1, wherein the sample is further combined with a plasma selected from the group consisting of (a) plasma known to be deficient for a particular clotting factor and (b) normal plasma.
6. The method of Claim 1, wherein the sample is from a subject that has been given heparin treatment.
7. The method of any of Claims 1-6, wherein thrombin enzymatic activity is measured.

8. The method of any of Claims 1-6, wherein clot formation is detected.

5 9. The method of Claim 1, wherein the soluble phospholipid consists essentially of a phospholipid selected from the group consisting of phosphatidylserine, phosphatidylhomoserine, phosphatidic acid, phosphatidylethanolamine, and a combination thereof.

10 10. The method of Claim 9, wherein the soluble phospholipid consists essentially of C6 phosphatidylserine.

11. The method of Claim 1, wherein the soluble phospholipid is added to a final concentration from about 4  $\mu$ M to about 2 mM.

15 12. The method of Claim 1, wherein the soluble phospholipid is in a dried form prior to combination with the sample.

20 13. The method of Claim 1, wherein the sample is a human blood or plasma sample.

14. The method of Claim 1, further comprising comparing the detected thrombin activity with a standard.

25 15. The method of Claim 1, wherein the contact activator is selected from the group consisting of kaolin, clay, silica, ellagic acid, celite, diatomaceous earth, glass beads, and a combination thereof.

30 16. A method of performing a clotting assay comprising:  
(a) combining a sample comprising blood or plasma with:  
(i) a soluble phospholipid;  
(ii) a contact activator; and  
(iii) calcium;

- (b) incubating the mixture of (a) above for a time and under conditions sufficient for clot formation.

17. The method of Claim 16, further comprising determining a clotting time for the sample.

18. The method of Claim 17, further comprising comparing the determined clotting time with a standard.

19. The method of Claim 16, wherein the sample is first combined with the contact activator, and is then combined with the soluble phospholipid and the calcium to initiate the clotting reaction.

20. The method of Claim 16, wherein the soluble phospholipid consists essentially of a phospholipid selected from the group consisting of phosphatidylserine, phosphatidylhomoserine, phosphatidic acid, phosphatidylethanolamine, and a combination thereof.

21. The method of Claim 16, wherein the sample is a human blood or plasma sample.

22. A method of detecting a deficiency in intrinsic clotting pathway activity comprising:

- (a) combining a sample comprising blood or plasma with:
  - (i) a soluble phospholipid;
  - (ii) a contact activator; and
  - (iii) calcium;
- (b) incubating the mixture of (a) above for a time and under conditions sufficient for clot formation;
- (c) determining a clotting time for the sample;
- (d) comparing the determined clotting time for the sample with a standard, wherein a prolonged clotting time as compared with

the standard is indicative of a deficiency in intrinsic clotting pathway activity.

23. A method of monitoring clotting time in a subject following  
5 heparin treatment comprising:
- (a) obtaining a sample comprising blood or plasma from a subject that has been given heparin treatment;
  - (b) combining the sample with:
    - 10 (i) a soluble phospholipid;
    - (ii) a contact activator; and
    - (iii) calcium;
  - (c) incubating the mixture of (b) above for a time and under conditions sufficient for clot formation;
  - (d) determining a clotting time for the sample, thereby monitoring  
15 clotting time in the subject following heparin treatment.

24. The method of Claim 23, further comprising comparing the determined clotting time for the sample with a standard.

- 20 25. The method of Claim 23, further comprising repeating (a) to (d) for at least one additional iteration.

26. The method of Claim 25, wherein the additional iteration(s) of the method are carried out at timed intervals.

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27. A method of evaluating Factor VII<sub>a</sub> activity comprising:
- (a) combining a sample comprising plasma with:
    - (i) a soluble phospholipid;
    - (ii) soluble tissue factor; and
    - 30 (iii) calcium;
  - (b) incubating the mixture of (a) above for a time and under conditions sufficient for thrombin activation; and

- (c) detecting thrombin activity, wherein thrombin activity is indicative of Factor VIIa activity in the sample.

5           28. A method of evaluating the activity of a clotting factor in the intrinsic clotting pathway comprising:

- (a) combining a sample comprising plasma with:
  - (i) a soluble phospholipid;
  - (ii) exogenous Factor X;
  - 10           (iii) activated phospholipid-dependent clotting factors other than Factor X that are dependent on the clotting factor being evaluated in the intrinsic clotting pathway or are required for activation of the clotting factor being evaluated; and
  - (iii) calcium;
- 15           (b) incubating the mixture of (a) above for a time and under conditions sufficient for Factor X activation to Factor X<sub>a</sub>; and
- (c) detecting Factor X<sub>a</sub> activity, wherein Factor X<sub>a</sub> activity is indicative of the activity of the clotting factor in the sample.

20           29. The method of Claim 28, wherein Factor X<sub>a</sub> activity is detected by a spectrophotometric assay for Factor X<sub>a</sub>.

            30. The method of Claim 28, wherein Factor X<sub>a</sub> activity is detected by detecting thrombin activity in the sample.

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            31. The method of Claim 28, wherein Factor X<sub>a</sub> activity is detected by detecting clot formation.

            32. The method of Claim 28, wherein the method is carried out to evaluate FVIII<sub>a</sub> activity, the method comprising:

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- (a) combining a sample comprising plasma with:
  - (i) a soluble phospholipid;
  - (ii) exogenous Factor X;

- (iv) exogenous Factor IX<sub>a</sub>; and
  - (iii) calcium;
- (b) incubating the mixture of (a) above for a time and under conditions sufficient for Factor X activation to Factor X<sub>a</sub>; and
- 5 (c) detecting Factor X<sub>a</sub> activity, wherein Factor X<sub>a</sub> activity is indicative of Factor VIII<sub>a</sub> activity in the sample.

33. The method of Claim 32, wherein the sample is further combined with thrombin.

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34. Use of a soluble phospholipid in an assay for clotting activity, wherein the soluble phospholipid is substituted for platelet membranes or synthetic membranes in the clotting factor assay.

15 35. The use of Claim 34, wherein the assay is of intrinsic pathway clotting activity.

36. In a method of performing an assay for clotting activity, the improvement comprising substituting a soluble phospholipid for platelet  
20 membranes or synthetic membranes in the assay.

37. The method of Claim 36, wherein the assay is an intrinsic pathway clotting assay.

25 38. An assay composition comprising:  
(a) a plasma sample;  
(b) a soluble phospholipid;  
(c) a reagent selected from the group consisting of (i) a contact activator, (ii) a soluble tissue factor, and (iii) exogenous Factor  
30 X; and  
(d) calcium;

wherein the assay composition does not contain exogenously added platelet membranes or synthetic membranes.

39. The assay composition of Claim 38, wherein the assay composition comprises a contact activator and further comprises Activated Protein C or a Protein C activator.

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40. The assay composition of Claim 39, wherein the assay composition further comprises Protein S depleted plasma.

41. The assay composition of Claim 38, wherein the assay composition comprises exogenous Factor X and exogenous Factor IX<sub>a</sub>.

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42. The assay composition of Claim 38, wherein the assay composition further comprises a plasma selected from the group consisting of (a) a plasma known to be deficient for a particular clotting factor and (b) normal plasma.

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43. A kit for evaluating clotting factor activity comprising:  
(a) a soluble phospholipid;  
(b) a reagent selected from the group consisting of (i) a contact activator, (ii) a soluble tissue factor, and (iii) a composition comprising Factor X.

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44. The kit of Claim 43 further comprising calcium.

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45. The kit of Claim 43 further comprising a standard.

46. The kit of Claim 43, wherein the kit comprises a contact activator and further comprises Activated Protein C or a Protein C activator.

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47. The kit of Claim 46, wherein the kit further comprises Protein S depleted plasma.

48. The kit of Claim 43, wherein the kit comprises a composition comprising Factor X and a composition comprising Factor IX<sub>a</sub>.

49. The kit of Claim 43, wherein the kit further comprises a plasma  
5 selected from the group consisting of (a) a plasma known to be deficient for a particular clotting factor and (b) normal plasma.